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Deep sequencing of Hevea brasiliensis *small RNAs*

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(A) Application of ethephon to a rubber tree tapping panel.

(B) Tapping panel of a healthy tree

(C) A sick tree showing tapping panel dryness (TPD)

Hevea brasiliensis is increasingly cultivated in marginal areas where environmental constraints are important. In addition to regular tapping, the management of some Hevea cultivars required ethephon application, which enhanced the laticifer metabolism and consequently the latex production. However, excessive metabolic activation can lead to Tapping Panel Dryness (TPD, Figure 1). This physiological disorder is a consequence of an oxidative stress in the latex cells, leading to membrane damages, flocculation of rubber particles and plugging of the latex vessels. TDP-induced annual loss of rubber production was estimated at 15-20%. Plant development, metabolism and response to environmental stresses are not only due to specific genetic programme at the mRNA and protein levels but also to the modulation of transcript accumulation or translation by small RNAs. We reported here the identification of 61 conserved miRNA families and their putative targets related to oxidative stress and TPD appearance.

> Identification of 61 conserved micro RNAs familly

Vitroplants and budded plants were subjected to various abiotic stresses (cold, salinity, water logging and deficit, light excess, hormonal treatment). Micro RNAs were cloned and sequenced with the Solexa technology. The unique small RNA sequences were mapped against the PMRD database (Zhang et al., 2010). From 670645 unique short sequences (17-38 nt), 18430 matched perfectly or near perfectly to known micro RNAs in other species. The accessions were classified into 61 micro RNA families among which 12 are predominant with more than 1000 reads such as miR 159, 166, 167, 169, 319, 390, 396, 398, 482.2, 2111 (Figure 2).

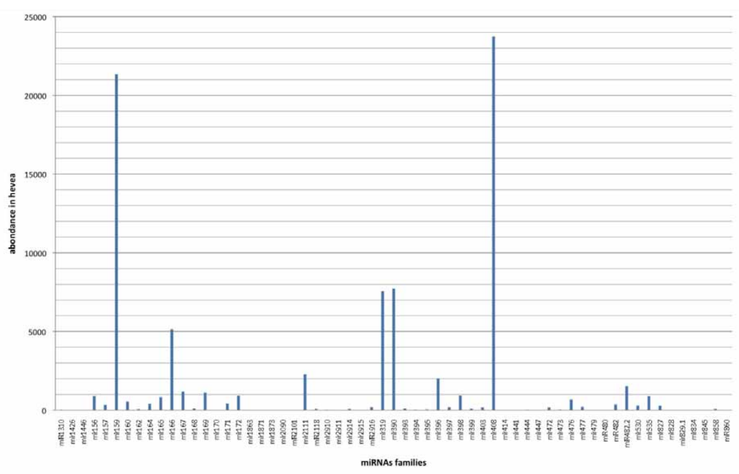

Figure 2: Abundance of 61 conserved micro RNA families in Hevea

> Computational prediction of micro RNA targets involved in oxidative stress and TPD

Putative micro RNAs targets were detected with Miranda toolbox comprised in LeARN pipeline by screening the *Hevea* transcript sequence database (Table 2). Among them, known targets were identified: ATP synthase/miR159, ABF2/miR159, Ja-ZIM-domain/miR159, ARF/miR160, AP2/miR172, CSD2/miR398, Metallothionein/miR396, Laccase/miR397-408. Interestingly, *Hevea* specific targets were also identified, five described for their differential expression between healthy and TPD tree (SSH80/miR156-157, SSH93/miR168, SSH100/miR1863 and SSH106/miR159), one involved in the cyanogenic potential (Linamarase P66/miR159) and two in rubber biosynthesis and stability (HMGR/miR159; Hevb13/miR159).

Tableau 2: List of the putative target involved in abiotic stress response, rubber biosynthesis and TPD appearance.

Mir family	Hevea contig	Alignment	Homology	Inhibition
MIR6446	Hevea_Rank_C5a2d5ooo1a6	miRNA 21 CACUACAGAAGAAAGAGUU 1 Target 1581 GTGTGTGTGTGTGTGTTTTCAA 1101	nucleoredoxin	Cleavage
MIR6446/157	Hevea_Rank_C5a1ooo1a2643	miRNA 20 ACUACAGAAGAAAGAGUU 1 Target 98 GTGTGTGTGTGTGTGTTTCAA 57	TPD related SSH 80	Cleavage
MIR6449	Hevea_Rank_C5a1d5ooo1a41	miRNA 20 CUACUAGAG-AAGUUAGUGG 1 Target 129 CAGUGTGTGTGTGAAGTCAAC 149	ATP synthase beta subunit mitochondrial	Cleavage
MIR6443	Hevea_Rank_C5a2d5ooo1a41	miRNA 21 AGCCCUGAGGGAAUUGAGUUU 1 Target 276 UGUCUGUCUCUCUCACCCUCAA 296	HMGR Hydroxymethyl glutaryl-CoA reductase	Cleavage
MIR6452	Hevea_Root_C5a1ooo1a5063	miRNA 21 UCUACGAAGUGAAGUUGUUU 1 Target 492 AAAUGUCUCUCUCUCUCAAA 502	cyanogenic beta-glucosidase PS6 expressed in soft bark	Cleavage
MIR6453	Hevea_Root_C5a1ooo1a5063	miRNA 20 UCUAGAUGGGAAGUUAGGUU 1 Target 84 AGAUCUUCCUUCUACCCAAA 103	TPD related SSH 126	Cleavage
MIR6459	Hevea_Rank_C5a1ooo1a8511	miRNA 22 AUCUGCCUCAAGUGAGUCACUU 1 Target 390 UGACUGGGUUCCCTCTCCUUCC 411	jasmonate Zim Domain protein 1(jaz1ddi)	Cleavage
MIR6452	Hevea_Leaf_C5a1d5ooo1a4	miRNA 21 ACACUCUAGUGGAAUCAGUUU 1 Target 451 UGUGAUGCUCCCUCAGUUCUA 471	Dev51 (DEVELop protein)	Cleavage
MIR6452	Hevea_Root_C5a1ooo1a1043	miRNA 21 UCCCCUAGGGAAU-UCAGUCU 1 Target 359 AGGGAGUUCCCCUUUAGUCCGC 330	ABA responsive element binding factor (ABF2)	Cleavage
MIR6459	Hevea_Rank_C5a1ooo1a64	miRNA 22 CUCCCUCGAGGGAAUCCAGUUU 1 Target 266 CAGGGAGCUCCCUCUUGUCCAC 287	Dead box RNA helicase like	Cleavage
MIR6460	Hevea_Rank_C5d1d5ooo1a41	miRNA 20 CCUUUAUGCCCCCUCGGUCUU 1 Target 276 GGCAUACAUGGAGCUCAGCCA 295	ABF (auxin responsee factor)	Cleavage
MIR6466	Hevea_Rank_C5a1d5ooo1a41	miRNA 20 CUUUUACCCCUCGGUCUUUU 1 Target 525 GGCAUGCAUGGAGCUCAGCCA 544	ABF (auxin responsee factor)	Cleavage
MIR6468	Hevea_Rank_C5a1ooo1a8163	miRNA 20 CUUUUACCCCUCGACCAGUCU 1 Target 860 GAAAUCCAAGCCUGGGUCCGA 879	potassium channel AFT1like	Cleavage
MIR6457	Hevea_Rank_C5a1ooo1a8163	miRNA 21 UACUUAGAUCAACCUCCUCAAUU 1 Target 77 AGGAGUCAUGGGGGGGUGGCA 97	Dead box RNA helicase like	Cleavage
MIR6458	Hevea_Rank_C5a1ooo1a8163	miRNA 21 AACGGCCUGGACCUGGGUCGCU 1 Target 880 CUCCGGACCUG-ACCAAGCGU 899	fatty acid desaturase (FAD)	Translation
MIR6472	Hevea_Rank_C5a1ooo1a41	miRNA 22 ACUAACUUGGGUGGUUCAGAUA 1 Target 344 UGAUUGACUUGACCAAUAUCA 385	zeaphytoiénoate reductase (zpr)	Cleavage
MIR6472	Hevea_454_rep_c22185	miRNA 23 CUACACUUGAAACUACCAUCAGU 1 Target 191 GAUGAUGCCCUGUGUGGCUGGC 213	AP2 (ORAP2-7,RAV)	Cleavage
MIR6472	Hevea_454_rep_c41090	miRNA 22 UACACCUAGAACUACUACCAGU 1 Target 69 AUGAUGAUCCUGAUGAUGGCGC 90	AP2 (ORAP2-20,RAV)	Cleavage
MIR6472	Hevea_454_rep_c14704	miRNA 22 UACACCUAGAACUACUACCAGU 1 Target 263 GUGUGGAUCCUGAUGAUGCCGC 284	AP2 (ORAP2-10,RAV)	Cleavage
MIR6472	Hevea_454_c45024	miRNA 22 AUACCUCCUAGUAUACCUAAUA 1 Target 285 UAUCUCAUC--CCAUCAAAUCCUU 305	AP2 (ORAP2-11,RAV)	Cleavage
MIR6472	Hevea_454_rep_c48825	miRNA 22 AGACCUCCUAGUAGUCCUAAUG 1 Target 1158 UUGUUGGCAUCAUCUAUAUCUUC 1129	AP2 (OBREP-III1C,OREB)	Cleavage
MIR6396	Hevea_Root_C5a2ooo1a92	miRNA 22 UAUUAAGUUCCUUCUACACCUU 1 Target 591 AUGAUCUAAAAAAUGUUCGGAA 612	WIN3/RIL	Cleavage
MIR6396	Hevea_Leaf_C5d1ooo1a84	miRNA 22 UUUUAAGUUCCUUCUACACCUU 1 Target 248 AGAAUUCAAUAAAUAUCUUUCAU 269	metallothionein like protein	Translation
MIR6436	Hevea_Rank_C5a1ooo1a1043	miRNA 20 UCAAUUUCUUUCUACACCUU 1 Target 606 AGAUCUAAGAAAGUUCUUCGA 625	cystein protease (CP1)	Cleavage
MIR6397	Hevea_Root_C5a1ooo1a1572	miRNA 21 AUAGUUUCUACUUUGAUCUACU 1 Target 75 UAUCAACUUUCACUUUCAUGA 95	TPD related SSH 88	Cleavage
MIR6397	Hevea_Root_C5d1ooo1a1572	miRNA 20 UAUUUCUACUUUGAUCUACU 1 Target 367 AUUAACUUCUCACUUUAUGA 386	Laccase	Cleavage
MIR6408	Hevea_Rank_C5a1d5ooo1a41	miRNA 18 UCCACUUGACUCUU-CUUU 1 Target 463 GGUGUGACUCUGGGAACAUA 481	ToTo-NOC chloroplastique	Cleavage
MIR6398	Hevea_Rank_C5a1ooo1a1043	miRNA 19 ACCACUUGACUCUUCUUUU 1 Target 912 UGGUGAACUCUGGGAACAUU 919	Laccase	Cleavage
MIR6477	Hevea_Leaf_C5d1ooo1a41	miRNA 20 CUCCUCUCUUCUCUCUCUCA 1 Target 1276 GAGAGAGGCUCUCAGAGAGAUA 1297	Ferredoxin NADP+reductase	Cleavage
MIR6420	Hevea_Rank_C5a1d5ooo1a8163	miRNA 20 CUCCAUGUCCACUCUUCAGU 1 Target 110 GAGGUCAGGAUCAAAUUGA 129	serine threonine phosphatase type 2A (PP2A)	Translation
MIR6420	Hevea_Rank_C5a1ooo1a1043	miRNA 20 CUCCAUGUCCACUCUUCAGU 1 Target 444 CAGGUUCAGGUCCAAAUGUC 463	Laccase	Translation
MIR6472	Hevea_Rank_C5a1ooo1a8163	miRNA 23 GUUUAAAUUCACCAUAUCUCUUA 1 Target 596 CAUGUUGACAUGGUGACGAGUCU 618	TPD related SSH 106	Translation
MIR6458	Hevea_Rank_C5a1ooo1a8163	miRNA 20 AGCUAGUGGUUGAUCCUGG 1 Target 950 UCCCCUCCUGACUAAGAGC 969	ferroline transporter	Cleavage

Micro RNAs precursors were identified in the *Hevea* clone PB260 sequences obtained from several organs with the 454 technology (Montoro et al., 2010) using LeARN pipeline (release 1.5.0, http://bioinfo.genopole-toulouse.prd.fr/LeARN; Noirot et al., 2008). Nine micro RNA families mapped against RNA sequences which displayed a stem-loop structure (Table 1). Among them, two miR precursor genes were identified for each micro RNAs family 159, 166, and 319/159, and three miR precursor genes for the micro RNA 408 family.

> Identification of precursors in the *Hevea* clone PB260 transcript sequence database

Tableau 1: List of micro RNA precursors identified in the *Hevea* clone PB260 transcriptome sequences

MIRNA name	N°acc	mature sequence	mature length	precursor length	EST	dG	Stem -loop structure	Homology (PMRD)
mir156	acc_462135	UUGACAGAAGAUAGAGA GC	19	119	FYGXE6O0 1:DJH4X_r1	76.16		ptc-mir156 j
mir159	acc_17403	UUUGGAUUGAAGGGAGC UCUA	21	221 214	GETTCZF0 1AWUSL_r1 CL19922Contig1_r1	118.67 120.38		rco-mir159
				216 212	CL1Contig10125_r1 GETTCZF0 16T133_r1	119.99 120.07		rco-mir159
mir166	acc_28021	UCGGA CCA-GGCUUCAUU CC	19	127 127	CL2671Contig1_r1 CL1Contig8281_r1	70.41 70.41		ptc-mir166i
	acc_150486	UCGGA CCA-GGCUUCAUU CCCCC	22	120	CL1Contig15359_r1 CL1Contig6849_r1	65.61		rco-mir166
mir319/15 9	acc_19786	UUGGACUGAAG GGA-GCUC CCU	21	221	FRZQES201D3 4W3_r1	125.04		ptc-mir319a
				194	FRZQES201D43A 4_r1	111.50		ptc-mir319a
mir396	acc_112787	UUCCACA GCUUCUUGUGAGCUG	21	154 155 154	CL1Contig13811_r1 CL1Contig9910_r1 CL4202Contig1_r1	87.55 84.20 87.55		ptc-mir396a
mir408	acc_10821	UGCAUU GCCUCUUC CUGG GC	20	127	GETTCZF01 8ZY7Y_r1	80.20		ptc-mir408
	acc_393516	AAGAUGGGGA ACAG GCAG AGCA	22	128 128	GETTCZF0 1AGGRG_r1 FYGXE601CVDV 9_r1	82.75 85.21		ptc-mir408
	acc_39581	ACUGGGA ACAG GCAGA GCAUGA	22	122 120	GETTCZF01C FXF3_r1 CL3908contig1_r1	72.30 74.92		ptc-mir408
mir476	acc_508145	UAAUCUUCUUUGCAAGUC	20	126	CL1Contig11471_r1	61.87		ptc-mir476a
mir2910	acc_251816	GAG CGAUUUGUCUGGUUAAUC	21	125 126	GETTCZF01CG KQ4_r1 FYGXE601 E0XGX_r1	55.28 55.97		pvu-mir2910
mir2916	acc_385724	AAGUUUGGC GGCU CGAAGA CGA	21	250	GETTCZF01C 6ZCT_r1	108.45		pvu-mir2916

Iconography : *Jean-Marie Eschbach, André Clément-Demange, Julie Leclercq*

Conclusion

All the putative targets of interest will be experimentally validated and the co-expression analysis of target genes and their corresponding micro RNAs will be carried out in order to have a better understanding on the control of the ROS-scavenging systems and the TPD onset in *Hevea brasiliensis*.

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